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VACTERL With the Mitochondrial NP 3243 Point Mutation

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The VACTERL association of vertebral, anal, cardiovascular, tracheo-esophageal, renal, and limb defects is one of the more common congenital disorders with limb deficiency arising during blastogenesis. The cause is probably heterogeneous; a molecular basis has not yet been defined. We report on a family in which a female infant with VACTERL was born in 1977 and died at age 1 month due to renal failure. Because her mother and sister later developed classical mitochondrial cytopathy associated with the A-G point mutation at nucleotide position (np) 3243 of mitochondrial (mt) DNA, we performed a molecular analysis of mt DNA in preserved kidney tissue from the VACTERL case. We discovered 100% mutant mt DNA in multicystic and 32% mutant mt DNA in normal kidney tissue. Mild deficiency of complex I respiratory chain enzyme activity was found in the mother's muscle biopsy. Other maternal relatives were healthy but had low levels of mutant mt DNA in blood. This is the first report to provide a precise molecular basis for a case of VACTERL. The differing tissue pathology depending on the percentage of mutant mt DNA suggests a causal connection between the mutation and symptoms. VACTERL, and this type of multicystic renal dysplasia, are new phenotypes for the np 3243 point mutation. The possibility of a mitochondrial disorder should be born in mind and also that VACTERL may occur as a first manifestation of a mutation that has been present for generations. This would have major implications for patient management and for genetic counselling regarding both the risk of recurrence and risk of other mitochondrial syndromes in affected families.

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KEY WORDS: VACTERL, MELAS, cardiomyopathy, mitochondrial disease

INTRODUCTION

VACTERL is a complex combination of vertebral, anal, cardiovascular, tracheo-esophageal, renal, and limb defects. It is one of the more common abnormalities with congenital limb deficiency defects, but the risk of recurrence is hard to estimate in individual cases. The association is almost certainly heterogeneous [Evans et al., 1989]; a phenotypically related form with hydrocephalus (VACTERL-H) has been defined as an x-linked mutation [Genuardi et al., 1993; Wang et al., 1993]. A noxious influence must take effect during blastogenesis to bring about VACTERL, but specific causes are not known. The A-G transition mutation at nucleotide position (np) 3243 of mitochondrial DNA (mt DNA) is most commonly associated with encephalopathy, lactic acidosis, and strokelike episodes (MELAS) [Goto et al., 1990; Pavlakis et al., 1984], but has recently also been recognized to cause diabetes mellitus [Kadowaki et al., 1994; van den Ouweland et al., 1992] and chronic progressive external ophthalmoplegia (CPEO) [Hammans and Morgan-Hughes, 1994]. In addition, mitochondrial mutations have been implicated in the cause of Parkinson disease [Wallace et al., 1992]. We detected the np 3243 mutation in preserved kidney tissue of a female child who died at age 1 month in 1977 with the VACTERL association. Her older sister developed MELAS and cardiomyopathy in 1979, and her mother presented with CPEO in 1991. To our knowledge, this is the first report to define a molecular basis of VACTERL and the first report of a selective molecular analysis of normal and pathological kidney tissue with the np 3243 mutation.

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MATERIALS AND METHODS

Biopsy materials from the left M. biceps brachii of patient III-1 was fixed in formalin, embedded with paraffin, and stained with haematoxilin and eosin (HE), Goldner's trichrome, and periodic acid-Schiff (PAS) for light microscopy (LM); frozen sections were stained with HE, trichrome, PAS, and oil red. The histochemical reactions used were NADH-TR, cytochrome c-oxidase, ATPase pH 9.4, phosphorylase, and acid phosphatase. Electron microscopy (EM) was performed from glutaraldehyde-fixed material stained with osmium tetroxide. HE stained paraffin sections of patient IV-2 were photographed (Fig. 1) and then processed further for molecular analysis. Biochemical studies of respiratory chain activity were performed according to accepted methods [Reichmann et al., 1986].

Genomic DNA was isolated from lymphoblasts or from 50 mg of frozen muscle tissue (patient III-1) for molecular analysis [Wallace et al., 1988]; HE-stained resin-fixed microscopic sections of kidney tissue of patient IV-2 were analysed after elution with xylol. The restriction endonucleases Bam HI or Pvu II were applied to 2 μg of genomic DNA for Southern analysis and electrophoretic separation performed on 0.5% agarose gel; the separated DNA fragments were placed on a nylon membrane by capillary blotting and hybridized with dioxygenin-marked highly purified mt DNA. The mt tRNA Leu(UUR) gene was amplified using primers A (hybridized with nucleotides 3007-3023 and B (hybridized with nucleotides 3717-3701) [Saiki et al., 1985]. Amplified DNA was purified and the restriction endonuclease Apa I applied. Resulting fragments were separated with agarose gel (1.5%), stained with ethidium bromide, and evaluated photographically.

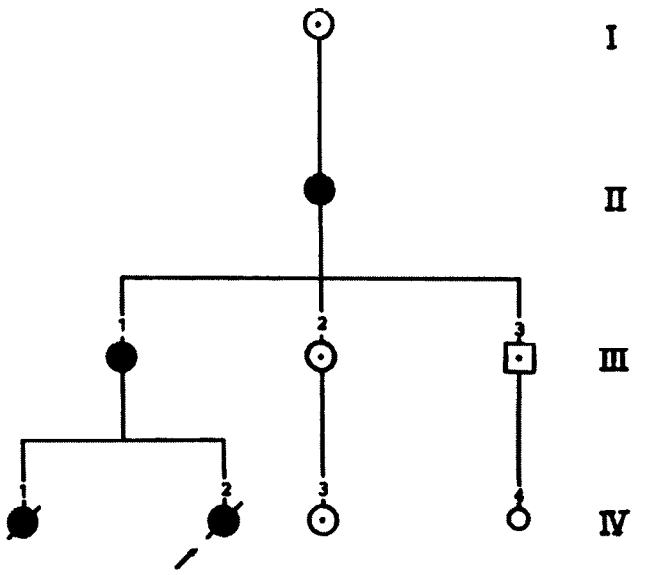

Fig. 1. Pedigree of the family under study. ● = symptomatic; ○ = prenatal test, no mutation; ⊡ ⊙ = mutation at np 3243 of mt DNA, asymptomatic. Clinical details in text.

CLINICAL REPORTS

Figure 1 shows the pedigree of the family under study. Relatives at risk for a maternally inherited disorder were examined after the np 3243 point mutation was detected in patient III-1, and the percentage of mutant mt DNA determined from lymphoblasts. Table I shows the percentage of mt DNA exhibiting the np 3243 point mutation on molecular analysis as well as patient age and symptoms. None had signs of myopathy, encephalopathy, cardiomyopathy, diabetes, or renal disease. However, patient II had mild neural deafness. Patient I remains healthy at age 91. Amniocentesis was performed in case IV-4 because of the mother's age, and amniotic-fluid cell cultures tested for the mutation were negative.

Patient IV-2

The proposita was born in 1977 at term by spontaneous vaginal delivery after a pregnancy complicated by bleeding in the third month. Birthweight was 2,680 g (tenth centile), length 46 cm (tenth centile), and head circumference (OFC) 35 cm (75th centile), Apgar index at birth 9. At birth she had hypoplasia of the left side of the face, absence of the left thumb and of the right thumb and index finger, shortness of the right arm, and bilateral radial deviation of the hands. An imperforate anus was noted as well as a rectovestibular fistula. There was a left parasternal 2/6 systolic murmur. Serum lactate was not measured. The infant was hypoglycemic (<25 mg%) at birth and glucose was substituted accordingly. Perioral cyanosis and a pathological ECG were noted. X-ray studies of the thorax at day 4 showed moderate global cardiac dilatation, normal lungs, and multiple rib anomalies. Spinal radiographs showed multiple cervical and thoracic vertebral wedging, fusion, and fission. There was aplasia of both radii, ulnar hypoplasia on the right, and bilateral aplasia of the radial digital rays. Recurrent urinary infections occurred during the next 2 weeks; an i.v. pyelogram and isotope nephrogram showed an enlarged, hydronephrotic right kidney and a silent left kidney, compatible with an "asymmetrical double kidney." The vestibular fistula was surgically enlarged to relieve impacted faeces causing urinary obstruction.

TABLE I. Percentage of mt DNA Exhibiting the np 3243 Point Mutation*

Patient	Age	Symptoms	% Mutant mt DNA
I	91 y	None	35 (blood)
II	66 y	Deafness	5 (blood)
III-1	43 y	CPEO	14 (blood); 77 (muscle)
III-2	42 y	None	3 (blood)
III-3	38 y	None	7 (blood)
IV-2	1 mo	VACTERL	32 (normal kidney); 100 (cystic kidney)
IV-3	19 y	None	17 (blood)
IV-4	Prenatal	None	0 (amniotic fluid cells)

* In brackets the respective tissue analyzed. Patient IV-1 was not tested.

Renal angiography performed on day 28 suggested an additional retroperitoneal space-occupying lesion. Subsequent laparotomy documented a cystic hydroureter. During the procedure the displaced right renal arterial supply was ligated irreversibly and an asymmetrical horseshoe kidney resected. Consequently the infant developed anuria and died in status epilepticus on day 31. Permission for autopsy was refused. Four HE-stained sections of the kidney were the only tissue conserved (Fig. 2). Molecular analysis in 1994 showed the np 3243 point mutation with 100% mutant met DNA in multicystic areas of the kidney specimen, and 32% mutant mt DNA in tissue appearing normal on LM.

Patient IV-1

The elder sister of the proposita was born in 1971 by forceps delivery due to breech presentation after an uneventful pregnancy. Birthweight was 3,750 g, length 51 cm. Early growth and development were reported as

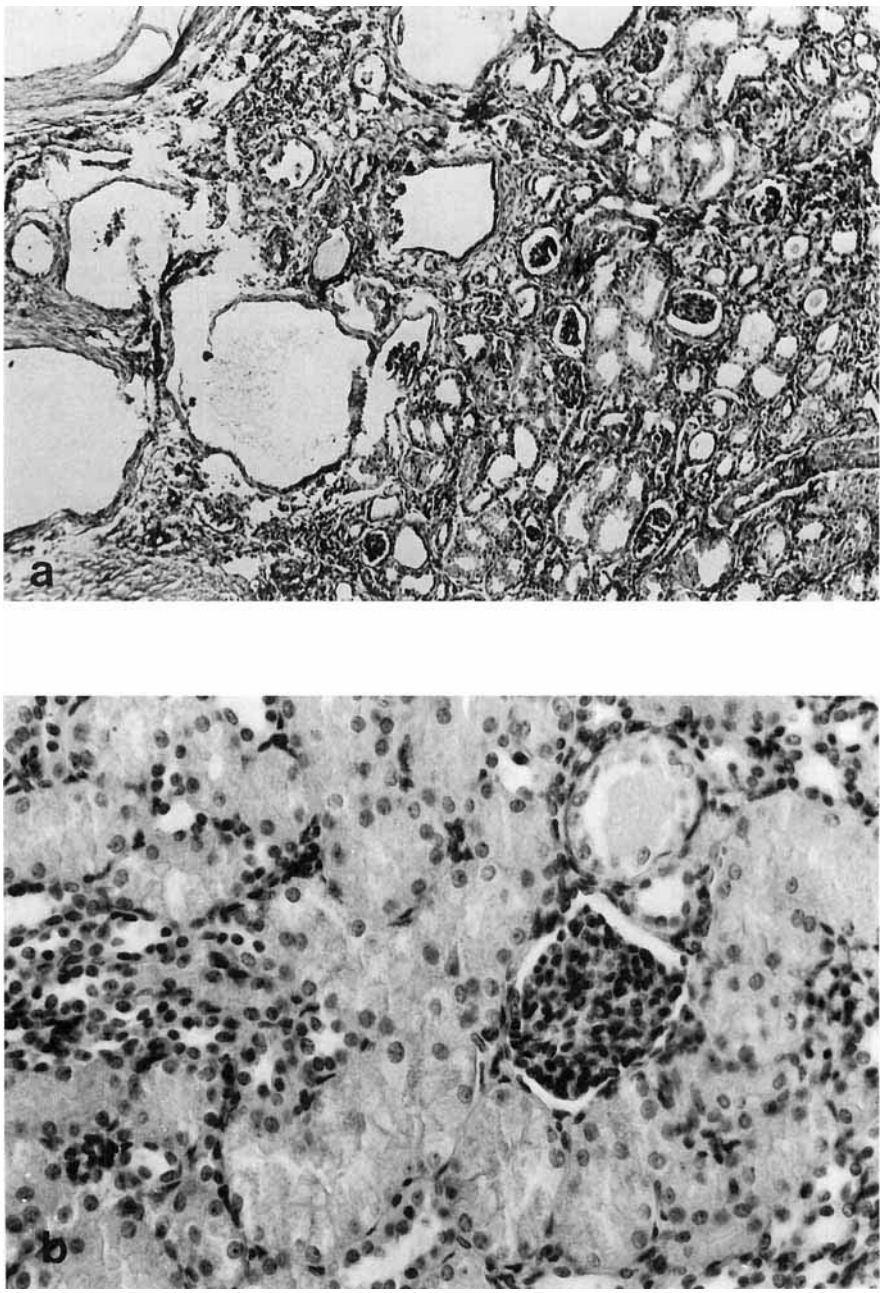

Fig. 2. HE-stained sections from the kidney (IV-2). **(a)** Low power micrograph shows renal tissue with multiple epithelium-lined cysts (×80). **(b)** High power micrograph from the renal cortex demonstrating a glomerulus and many cross-sectioned, often collapsed tubules. Many epithelial cells of proximal tubules reveal an eosinophilic fine granular cytoplasm (×500). Patient identification numbers refer to Figure 1.

normal. At age 9 years, she was seen after a seizure beginning on the right side. Slight neural deafness was the only neurological abnormality; mild glomerular proteinuria was noted. Electroencephalography (EEG) demonstrated left occipital slowing and paroxysmal discharges. Hearing loss progressed and further development was markedly retarded. Seizures were refractory to multiple anticonvulsant drugs. Focal headaches with hemianopia or hemiparesis were first classified as migrainous episodes, but visual field defects resolved incompletely. Lactic acidemia was noted repeatedly. In 1985 a severe cardiomyopathy was diagnosed. A cardiac biopsy showed accumulations of deformed mitochondria of irregular size with concentric cristae on EM, permitting a diagnosis of MELAS with mitochondrial cardiomyopathy. Neurological evaluation at age 16 showed moderate dementia, mild ataxia, bilateral incomplete hemianopia, severe deafness, and generalized proximal myopathy. The patient died 1 year later of heart failure. Autopsy was not done.

Patient III-1

The proposita's mother, born 1951, noted mild exertion-dependent weakness in her thirties, but was first examined in 1991 after developing frank myopathy. She had bilateral ptosis and external ophthalmoparesis, moderate proximal tetraparesis and mild neural deafness, and resting lactic acidemia. A muscle biopsy demonstrated multiple ragged red fibres and pathological mitochondrial ultrastructure on EM (Fig. 3). Biochemical analysis of respiratory chain function showed mild Complex I deficiency (32.4 U/g muscle; normal: 48 ± 9.4 U/g) and otherwise normal respiratory chain enzyme activity. Analysis of mt DNA demonstrated the A-G transition mutation at np 3243 (14% mutant mt DNA in blood, 77% mutant mt DNA in muscle). EEG, evoked potentials and cerebral magnetic resonance imaging showed no central nervous system (CNS) involvement. Echocardiography was normal. Glucose tolerance tests were normal. Muscle strength and exertion tolerance have improved since ubiquinone substitution was started (150 mg/d) and lactate levels on exertion tests decreased. Cerebral status remains normal on follow-up.

DISCUSSION

This pedigree is interesting for several reasons. First, the mutation was already present in at least two generations without causing severe symptoms, and one of the carriers is still healthy at 91. Patient IV-1 had the MELAS syndrome complicated by severe mitochondrial cardiomyopathy. A large pedigree with cardiomyopathy and a different point mutation (np 3260) in the same gene has been described [Zeviani et al., 1991], but cardiomyopathy has seldom been reported in MELAS cases. Patient III-1, again, has no CNS disease, but CPEO as mainly noted with deletions of mt DNA, and recently recognized with the np 3243 point mutation [Hammans and Morgan-Hughes, 1994]. This is the first report associating a mitochondrial mutation with VACTERL, which constitutes 8% of congenital limb deficiency defects associated with other malformations [Evans et al., 1994]. Most cases of VACTERL are sporadic [Evans et al., 1989; Evans et al., 1994], but a phenotypically similar x-linked form (VACTERL-H) has been described [Genuardi et al., 1993; Wang et al., 1993]. The general risk of recurrence is estimated at ≤2%, but is greater in VACTERL-H [Evans et al., 1989]. VACTERL-H carries a graver prognosis and is the only subgroup to have been set apart as a possible discrete syndrome. We could not ascertain whether the CNS was involved in the case presented here, as seizures were only terminal events and brain imaging or autopsy were not performed. There is considerable clinical overlap among VACTERL, trisomy 13, and trisomy 18 [Evans et al., 1989], and in some cases of VACTERL-H increased chromosome breakage has suggested a link to Fanconi anemia [Iafolla et al., 1991; Poole et al., 1992; Porteous et al., 1992; Wang et al., 1993]. No further evidence has yet implicated a specific molecular defect, but Beemer and co-workers [1990] were able to demonstrate a peroxisomal disorder in two sibs with VACTERL findings and hydrocephalus.

The occurrence of VACTERL in this family could be coincidental given the frequency of the association, but we propose that mitochondriopathy might have played a causal role. This could either be direct, or as a synergistic influence of oxidative disturbance on an unknown dysmorphogenetic mechanism. A coincidence would be more likely if mitochondrial disease in patient IV-2 had been as mild as in all other relatives at the time. However, patient IV-2 was severely affected with no wild-type mt DNA demonstrable in multicystic areas of the resected kidney, and 68% wild-type mt DNA in normal-appearing areas. The renal disorder, at least, is likely to be a direct consequence of mitochondriopathy. We have reported several cases of nephropathy in another MELAS pedigree [Damian et al., 1995], and this is the first case in which the np 3243 point mutation has been quantified selectively in normal and in multicystic areas of kidney in the same patient. Genotype and phenotype in the MELAS mutation correlate only broadly, but severely affected children with early onset MELAS tend to have a high percentage of mutant mt DNA [Damian et al., 1995]. Severely impaired oxidative metabolism of the embryo may conceivably disturb blastogenesis, the period implicated in VACTERL. The incidence of fetal death and perinatal mortality in families with mitochondrial disease is not clear, and there is little information on pathological or molecular findings in offspring of patients with this point mutation. Maternal factors also could be significant, even though the mother's CPEO manifested years later and diabetes was ruled out. The consequences of maternal oxidative malfunction and lactic acidemia on the embryo are hard to estimate. Detailed studies of aborted fetuses and nonviable infants in pedigrees with the mutation are needed to determine the frequency of VACTERL or related defects. Pedigrees with recurrent VACTERL or VACTERL-H compatible with maternal inheritance should be reappraised to identify characteristics of patients or their mothers suggesting mitochondriopathy.

If further cases of VACTERL could be linked to the np 3243 mutation, the risk of recurrence of VACTERL or other phenotypes such as MELAS, CPEO, cardiomyopathy or diabetes in such families would have a major impact on genetic counselling. A detailed family study and analysis of mt DNA is necessary even in clinically normal maternal relatives of MELAS patients. Patient management includes regular assessment of cerebral, cardiac, muscular and renal function, because of the potential for late manifestation. Even clinically normal carriers of the mutation need to be monitored for early signs of metabolic deterioration, such as lactic acidemia,

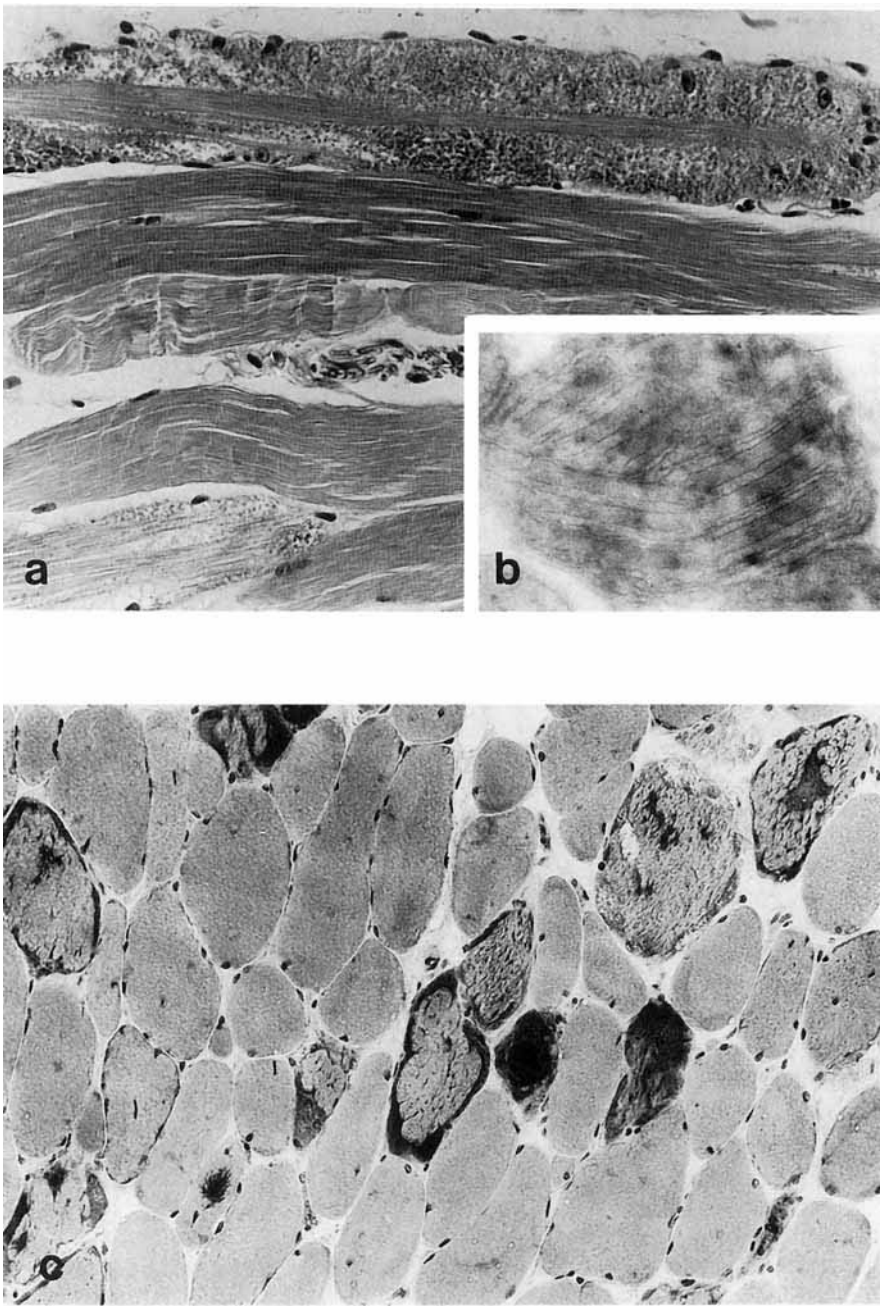

Fig. 3. Muscle biopsy demonstrating myopathy with ragged red fibers (III-1). **(a)** Trichrome (Goldner) stain reveals masses of (in the original paraffin section red coloured granula (i.e., mitochondria) concentrated in the lateral sarcoplasm accompanying bundles of myofibrils (×330). **(b) Inset:** Electron micrograph of an abnormal, enlarged mitochondrium of a muscle fiber with groups of rectangular paracristalline inclusions (×60,000). **(c)** Cryostat section of frozen muscle tissue showing numerous ragged red fibers with accumulation of (red stained, here dark) mitochondria especially in the lateral sarcoplasm. Further myopathic changes were irregular fiber caliber and internal nuclei (Goldner trichrome, transverse section, ×200).

exercise interolance, or "migraine." The blood test for the np 3243 point mutation is a useful screening tool, but negative results in at-risk persons should be confirmed by molecular analysis of a muscle biopsy. Experience of mt DNA analysis in amniotic fluid cell cultures is at present too limited to know whether the results are reliable enough for prenatal testing. Furthermore, the consequences arising from a positive result are unclear, as carriers of the mutation can remain oligosymptomatic or clinically unaffected lifelong.

In conclusion, VACTERL is a new phenotype for the np 3243 point mutation of mt DNA and may be the first manifestation of mitochondriopathy in clinically normal families. Selective molecular analysis of mt DNA in different areas of the kidney suggested a direct relationship between multicystic dysplasia and high percentage of mutant mt DNA. Clinical or laboratory findings suggesting oxidative dysfunction in children with VACTERL or their mothers call for further investigation, including analysis of mt DNA. In genetic counselling a potential risk of recurrence should be born in mind, if mitochondrial pathology has not been ruled out.

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REFERENCES

Beemer FA, Wanders RJA, Schutgens RBJ (1990): VACTERL and hydrocephalus. Am J Med Genet 37:425–426.

Damian MS, Seibel P, Reichmann H, Schachenmayr W, Laube H, Bachmann G, Wassill KH, Dorndorf W (1995): Clinical spectrum of the MELAS mutation in a large pedigree. Acta Neurol Scand 92:403–409.

Evans JA, Stranc LC, Kaplan P, Hunter AGW (1989): VACTERL with hydrocephalus: Further delineation of the syndrome(s). Am J Med Genet 34:177–182.

Evans JA, Vitez M, Czeizel A (1994): Congenital abnormalities associated with limb deficiency defects: A population study based on cases from the Hungarian Congenital Malformation Registry. Am J Med Genet 49:52–66.

Genuardi M, Neri G, Chiurazzi P, Capelli A (1993): X-linked VACTERL with hydrocephalus: The VACTERL-H Syndrome. In Opitz JM (ed): "Blastogenesis: Normal and Abnormal." New York: John Wiley & Sons, pp 227–233.

Goto Y, Nonaka I, Horai S (1990): A mutation in the tRNA (Leu (UUR)) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature 348:651–653.

Hammans SR, Morgan-Hughes JA (1994): Mitochondrial myopathies: clinical features, investigation, treatment and genetic counselling. In Shapira AHV, DiMauro S (eds): "Mitochondrial Disorders in Neurology." Oxford: Butterworth-Heinemann, pp 49–74.

Iafolla AK, McConkie-Rosell A, Chen YT (1991): VATER and hydrocephalus: distinct syndrome? Am J Med Genet 38:46–51.

Kadowaki T, Kadowaki H, Mori Y, Tobe K, Sakuta R, Suzuki Y, Tanabe Y, Sakura H, Awata T, Goto Y, Hayakawa T, Matsuoka K, Kawamori R, Kamada T, Horai S, Nonaka I, Hagura R, Akanuma Y, Yazaki Y (1994): A subtype of diabetes mellitus associated with a mutation of mitochondrial DNA. N Engl J Med 330:962–968.

Pavlakis SG, Phillips PC, DiMauro S, DiVivo DC, Rowland LP (1984): Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS): A distinctive clinical syndrome. Ann Neurol 16:481–488.

Poole SR, Smith ACM, Hays T, McGavran L, Auerbach AD (1992): Monozygotic twin girls with congenital malformations resembling Fanconi anemia. Am J Med Genet 42:780–784.

Porteous MEM, Cross I, Burn J (1992): VACTERL with hydrocephalus: One end of the Fanconi anemia spectrum of anomalies? Am J Med Genet 43:1032–1034.

Reichmann H, Rohkamm R, Zeviani M, Servidei S, Ricker K, DiMauro S (1986): Mitochondrial myopathy due to complex III deficiency with normal reducible cytochrome b concentration. Arch Neurol 43:83–86.

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985): Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1354.

van den Ouweland JMW, Lemkes HHPJ, Ruitenbeek W, Sandkuijl LA, de Vijlder MF, Struyvenberg PAA, van de Kamp JJP, Maassen JA (1992): Mutation in mitochondrial tRNA Leu(UUR) gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. Nature Genet 1:368–370.

Wallace DC, Zheng XX, Lott MT, Shoffner JM, Hodge JA, Kelly RI, Epstein CM, Hopkins LC (1988): Familial mitochondrial encephalopathy (MERRF): genetic, pathophysiological, and biochemical characterization of a mitochondrial DNA disease. Cell 55: 601–610.

Wallace DC (1992): Diseases of the mitochondrial DNA. Annu Rev Biochem 61:1175–1212.

Wang H, Hunter GWA, Clifford B, McLaughlin M, Thompson D (1993): VACTERL with hydrocephalus: Spontaneous chromosome breakage and rearrangement in a family showing apparent sex-linked recessive inheritance. Am J Med Genet 47:114–117.

Zeviani M, Gellera C, Antozzi C, Rimoldi M, Morandi L, Villani F, Tirati C, Di Donato S (1991): Maternally inherited myopathy and cardiomyopathy: Associated with mutation in mitochondrial DNA tRNA Leu(UUR). Lancet 338:143–147.